Supplementary Information for

Ultra-Sensitive pH Control of Supramolecular Polymers and Hydrogels: pK_a Matching of Biomimetic Monomers

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General Information

Assemblies were formed by adding **CyCo6** and/or **CyCo4** to a solution containing an equivalent amount of **TAP** and 200 mM sodium phosphate buffered at pH 7; pH was adjusted with NaOH or HCl. Unless otherwise noted, assembly solutions were equilibriated and analyzed at 20 °C. **CyCo4** and **CyCo6** purity were determined by NMR and LCMS. NMR analysis for molecular characterization and precipitate composition was performed in DMSO-d_6 on a Varian Mercury 400 MHz NMR. HRMS was performed using a Waters Synapt G2. pH measurements were taken with a VWR 8100 pH meter equipped with an InLab semi-micro combination electrode.

Spectroscopic analysis of the assemblies was performed using UV-vis and ^{1}H NMR. UV-vis analysis was carried out on an Agilent 8453 spectrophotometer equipped with an 89090A temperature controller. Cells of different path lengths (0.1 and 0.01 mm) were used depending on the concentration of the sample to maintain an optical density below 1.2. ^{1}H NMR spectra were collected on a Bruker DRX-500 500 MHz NMR and were the sum of 64 transients. Assembly solutions investigated by NMR contained 90% H_2O and 10% D_2O and were observed using the WATERGATE pulse sequence. Cytosine was used as an internal standard at a starting concentration of 25 mM, cytosine did not show any indication of interacting or being incorporated within the assemblies.

AFM imaging was performed on freshly cleaved mica that was pre-activated with $MgCl_2$ with 1-2 h incubation. The mica substrate was rinsed with water and dried under N_2. Solutions containing the assemblies were incubated on ice just prior to deposition. A 2 µl sample of the assembly solution was spin coated for 30 s at 2500 rpm and dried with N_2 gas. AFM imaging was performed with a Nanoscope IIIa (Digital Instruments) in tapping mode, using Si tips (Vistaprobes, 48 N/m).

Rheological measurements were carried out on a Physica MCR 501 rheometer (Anton Paar). The storage modulus, G', and loss modulus, G'', were measured in oscillatory tests at a constant angular frequency of 1 rad/s while sweeping the strain. Frequency scans were performed under a strain of 1.0%. All measurements were temperature controlled with a Peltier plate at 20 °C.

Materials and Synthesis

2,4,6-triaminopyrimidine (**TAP**) was purchased from Acros Organic and was used as received. Synthesis of 1-(5-Carboxypentyl)-1,3,5-triazin-2,4,6-trion (**CyCo6**) and 1-(3-Carboxypropyl)-1,3,5-triazin-2,4,6-trion (**CyCo4**) was performed by following/modifying the procedure reported by Hager *et al.*, (*see reference 17*).

CyCo6: ^{1}H NMR (500 MHz, DMSO-d_6): δ = 1.27 (m, 2H; CH_2), 1.50 (m, 4H; CH_2), 2.19 (t, J = 7.5 Hz, 2H; CH_2CO), 3.61 (t, J = 7.5 Hz, 2H; CH_2N), 11.63 ppm (brm, NH); ^{13}C NMR (125 MHz, DMSO-d_6): δ = 24.1, 25.6, 27.05, 33.5, 40.2, 148.6, 149.8, 174.4 ppm; HRMS *m/z* calcd. for $[M - H]^-$, 242.0777; found, 242.0785.

CyCo4: ^{1}H NMR (500 MHz, DMSO-d_6): δ = 1.75 (m, 2H; CH_2), 2.24 (t, J = 7.5 Hz, 2H; CH_2CO), 3.68 (t, J = 7 Hz, 2H; CH_2N), 11.63 ppm (brm, NH); ^{13}C NMR (125 MHz, DMSO-d_6): δ = 23.2, 31.4, 40.5, 149.2, 150.44, 174.5 ppm; HRMS *m/z* calcd. for $[M - H]^-$, 214.0464; found, 214.0451.

Supplementary Figures

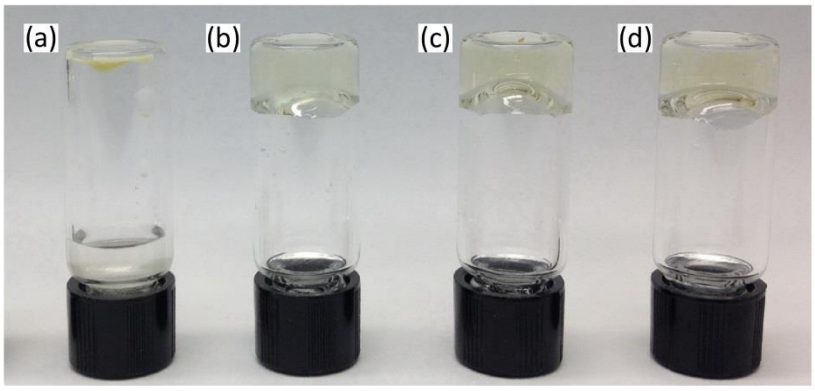


Fig. S1 Gel-inversion tests of various **TAP**-**CyCo4** assemblies. Each vial contains 20 mM **TAP** and 20 mM modified-**Cy** species buffered at pH 7 at 20 °C. Solutions contained (a) **CyCo4** and **TAP** (precipitate, no gelation), (b) same as (a), but with an additional 1 M NaCl which shows evidence of precipitation within 5 minutes, (c) **TAP**, **CyCo4** and **CyCo6** in a 1:0.5:0.5 ratio, respectively (no additional NaCl) which begins to precipitate after 30 minutes, and (d) **TAP**, **CyCo4** and **CyCo6** in a 1:0.8:0.2 ratio, respectively, which begins to precipitate after 5 minutes. Note that the rate of precipitation is decreased at lower temperature, for example precipitation began after 2 hours for gels described in (c) when stored at 4 °C.

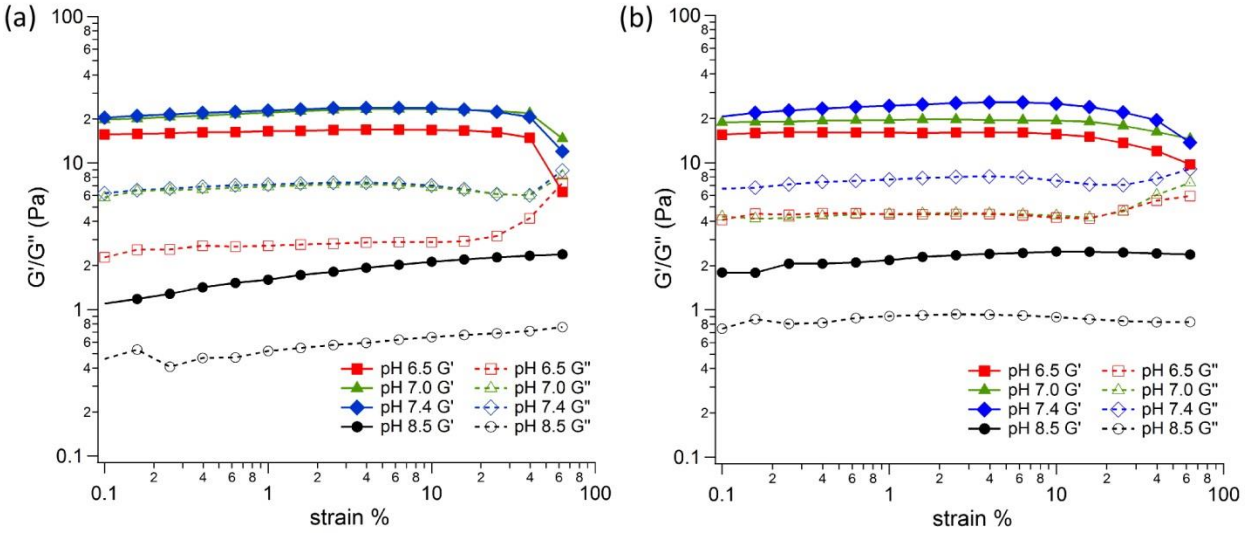


Fig. S2 Strain sweeps for gels prepared using (a) **TAP**-**CyCo6** and (b) **TAP**-**CyCo4**-**CyCo6** (1:0.66:0.33 ratio) at pH 6.5, 7.0, 7.4 and 8.5. Solutions were 40 mM in both **TAP** and **CyCo** monomer (1.4% by weight in total monomer).

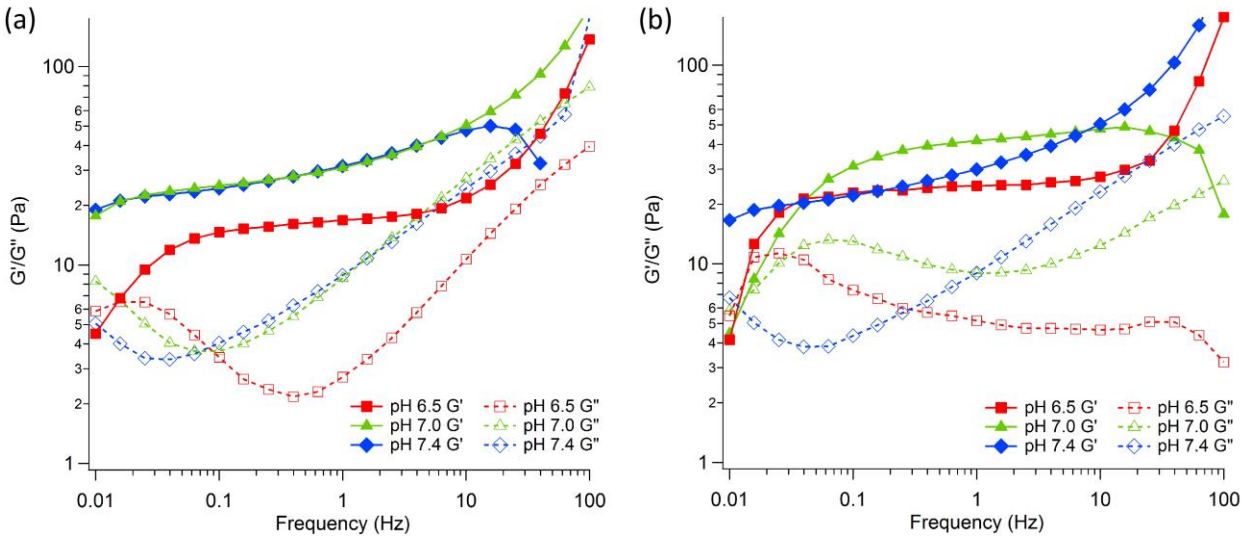


Fig. S3 Frequency sweeps for gels prepared using (a) **TAP**-**CyCo6** and (b) **TAP**-**CyCo4**-**CyCo6** (1:0.66:0.33 ratio) at pH 6.5, 7.0, 7.4 and 8.5. Solutions were 40 mM in both **TAP** and **CyCo** monomer (1.4% by weight in total monomer).

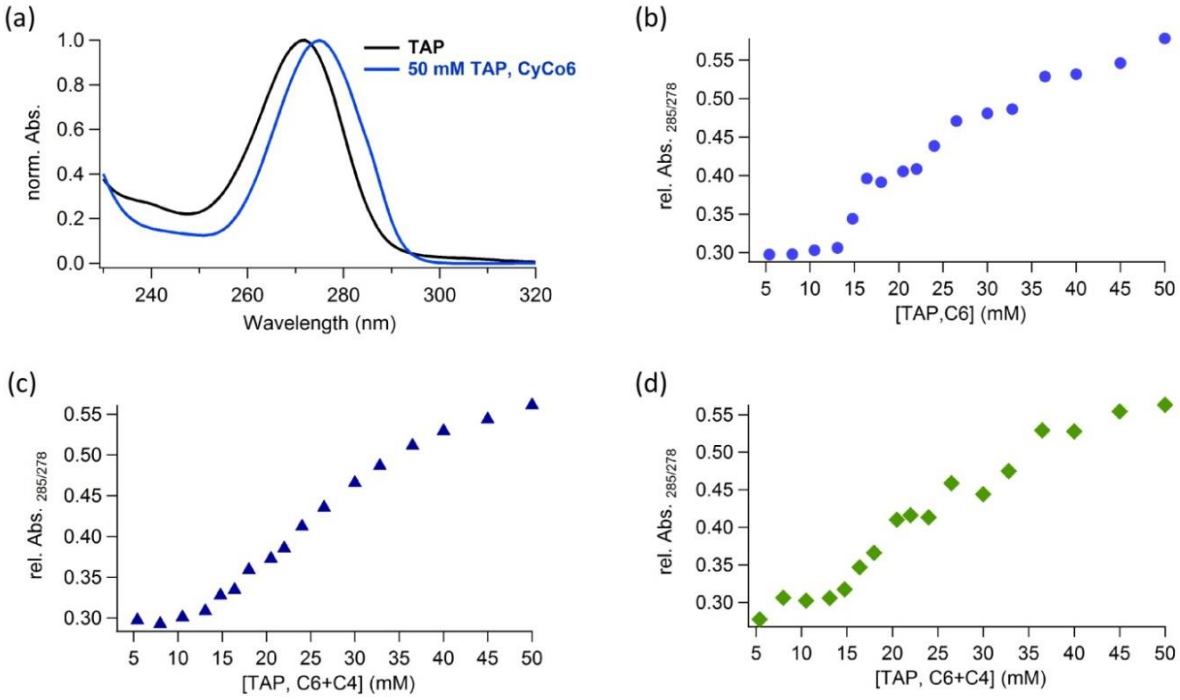


Fig. S4 UV absorption analysis of **TAP**, **CyCo4** and **CyCo6** at various concentrations. (a) Spectra of **TAP** alone (black) and **TAP** in a solution with **CyCo6** at 50 mM in each monomer (blue) where the absorption maxima have been normalized to unity. (b-d) Plot of the absorption ratio of A285 nm/A278 nm as a function of monomer concentration in (b) 1:1 mixtures of **TAP:CyCo6**, (c) 1:0.5:0.5 mixtures of **TAP**:**CyCo4**:**CyCo6**, and (d) 1:0.33:0.66 mixtures of **TAP**:**CyCo4**:**CyCo6**. All solutions were buffered at pH 7 and held at 20 °C.

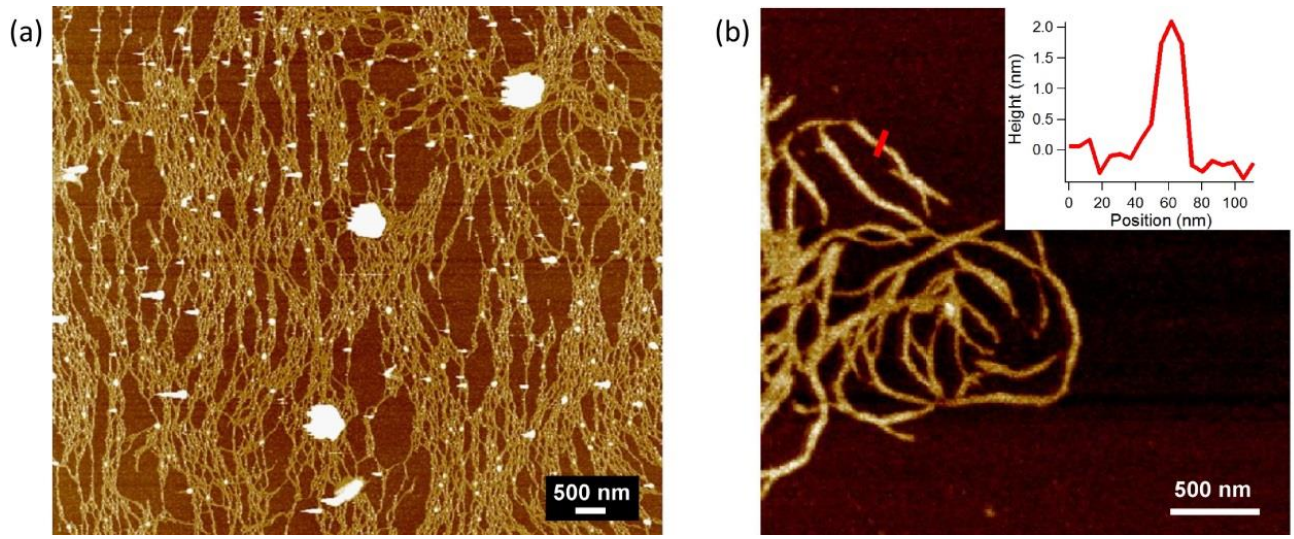


Fig. S5 AFM topographic images of **TAP-CyCo** supramolecular structures. (a) Sample containing 15 mM **TAP** and **CyCo6**. (b) Sample containing 15 mM **TAP**, 5 mM **CyCo4**, 10 mM **CyCo6** (1:0.33:0.66 ratio of **TAP**:**CyCo4**:**CyCo6**). Insert shows height profile delineated by the red line in the main panel.

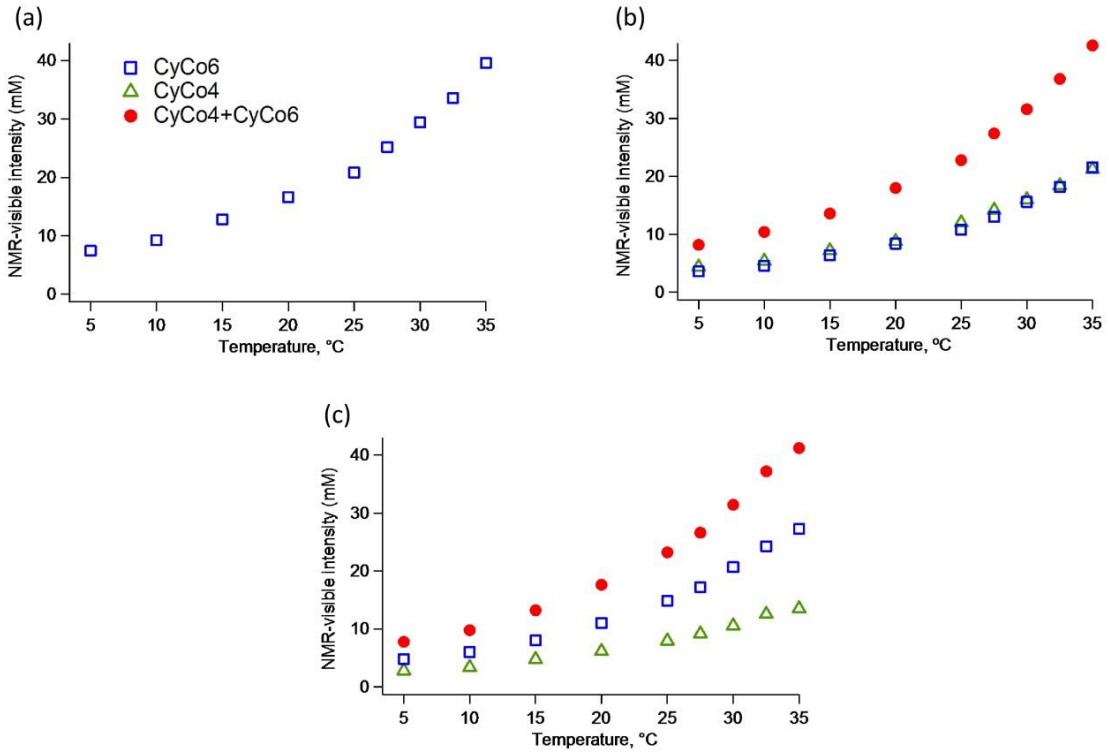


Fig. S6 Plots of the apparent solution-phase concentrations (equivalent to the MAC) of **TAP**-**CyCo** assemblies vs temperature. All solutions contained 40 mM **TAP** and 40 mM **CyCo4**+**CyCo6** at (a) 1:0:1, (b) 1:0.5:0.5 or (c) 1:0.33:0.66 molar ratios of **TAP**:**CyCo4**:**CyCo6**. Solutions contained 40 mM **TAP** and 40 mM **CyCo4**+**CyCo6**.

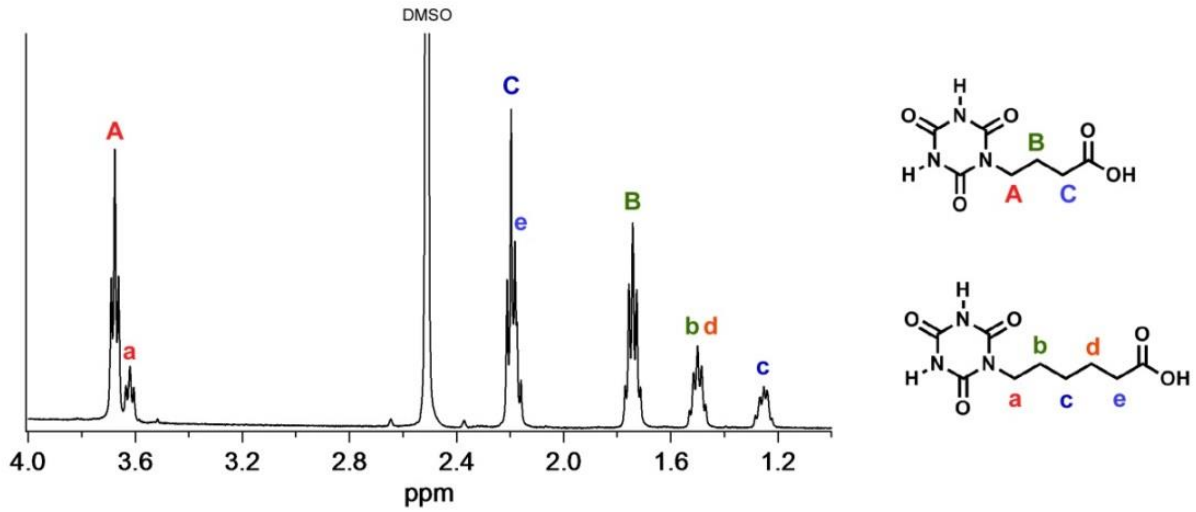


Fig. S7 ^{1}H NMR spectrum of precipitated material collected from a solution originally containing a 1:0.5:0.5 ratio of **TAP**:**CyCo4**:**CyCo6**. **TAP** and **CyCo4**+**CyCo6** were originally 40 mM in 200 mM sodium phosphate buffer, pH 7. Precipitation from the gel phase began after two hours, resulting in complete gel collapse over the course of 24 hours. The NMR spectrum of the precipitate dissolved in DMSO reveals precipitate enrichment in **CyCo4**, with a 4:1 ratio of **CyCo4**:**CyCo6**. Resonance assignments indicated on the **CyCo4** and **CyCo6** chemical structure were used to determine the ratio of both molecules in the isolated precipitate.

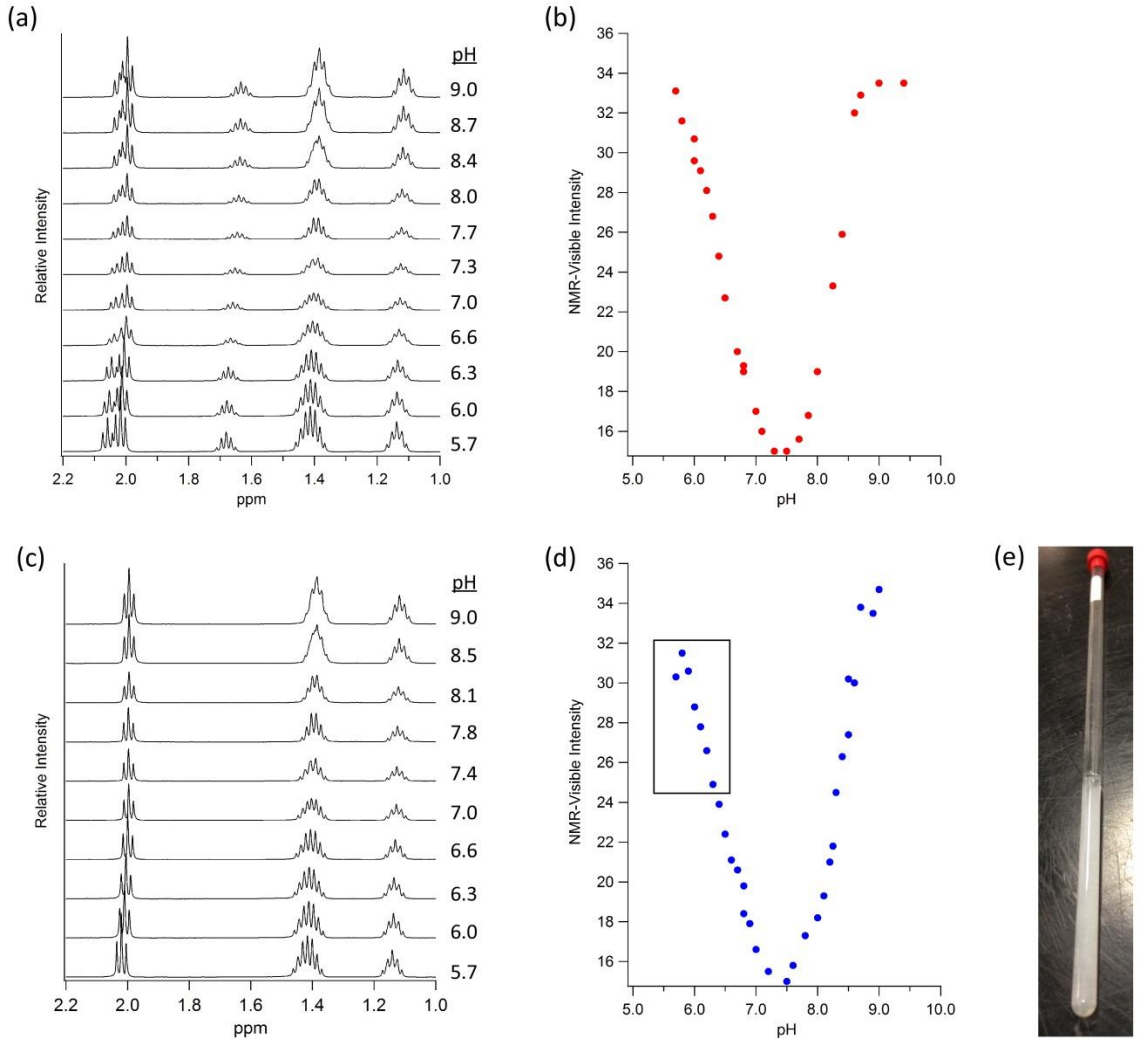


Fig. S8 Effect of pH on **TAP-CyCo** assemblies as followed by ^{1}H NMR. (a) Representative spectra of a solution containing a 1:0.33:0.66 ratio of **TAP**:**CyCo4**:**CyCo6**, at 35 mM in **TAP** and **CyCo4**+**CyCo6**. (b) Plot of the apparent solution-phase concentration as determined from the NMR experiment shown in (a) of **CyCo6** + **CyCo4** vs pH, which provides the MAC as a function of pH. (c) Representative spectra of a solution containing a 1:1 ratio of **TAP**:**CyCo6** originally at 40 mM in each monomer. (d) Plot of the apparent solution-phase concentration as determined from NMR experiment shown in (c) of **CyCo6** vs pH. Note that the box in left corner of plot indicates samples that contained **TAP**-**CyCo6** precipitate. (e) NMR tube containing a solution with a white precipitate of **TAP** and **CyCo6** at pH 6.5 from the titration experiment.

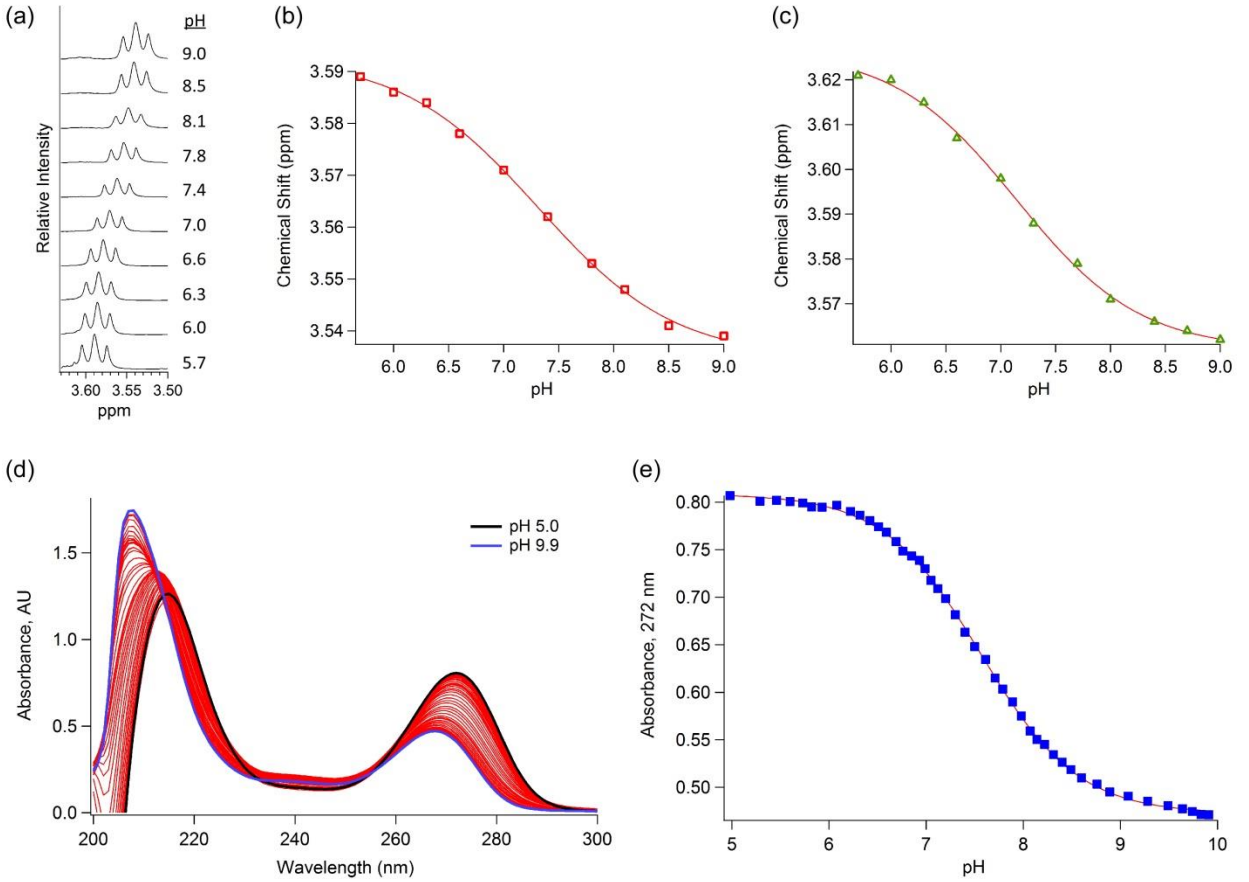
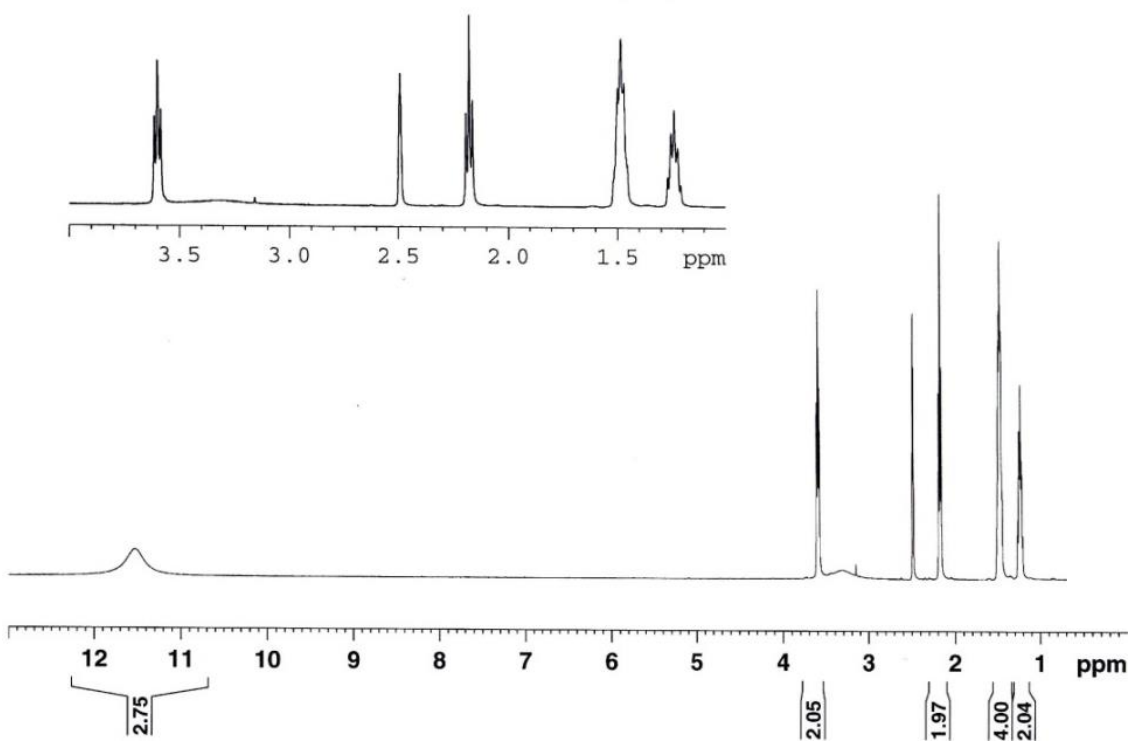
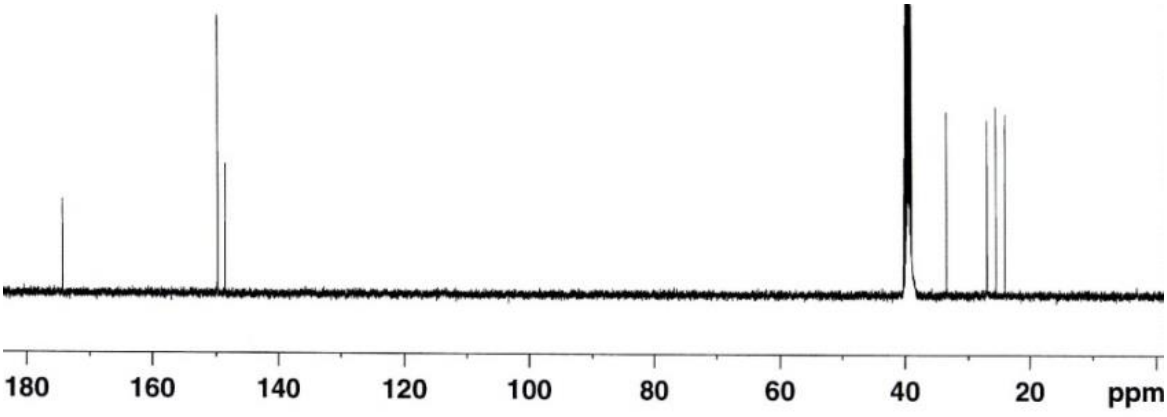


Fig. S9 pK_a determination of the **Cy** heterocycle for **CyCo6** and **CyCo4** by ^1H NMR and for **TAP** by UV-vis in 200 mM sodium phosphate buffer at 20 °C. (a) Representative ^1H NMR spectra of the methylene proton peak used to determine the pK_a of **CyCo6**. (b) Plot of **CyCo6** methylene peak position as a function of pH. (c) Plot of **CyCo4** methylene peak position as a function of pH. (d) UV-vis spectra of **TAP** (50 μM) at various pHs between 5.0 and 9.9. (e) Change in absorbance of **TAP** at 272 nm (where the protonated form of **TAP** absorbs maximally) as a function of pH. Sigmoidal fits (shown) reveal pK_as of 7.3 for **CyCo6**, 7.2 for **CyCo4** and 7.5 for **TAP**.

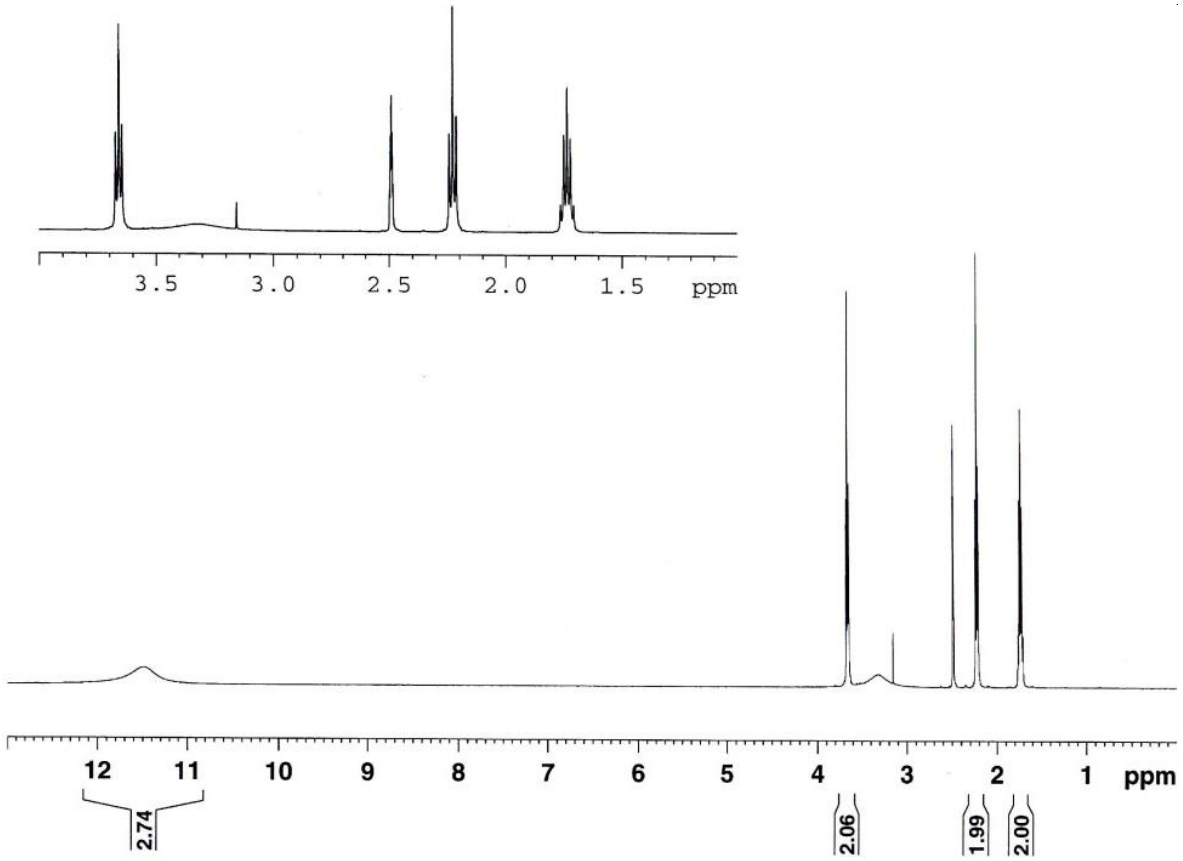
^{1}H and ^{13}C Spectra of CyCo Compounds



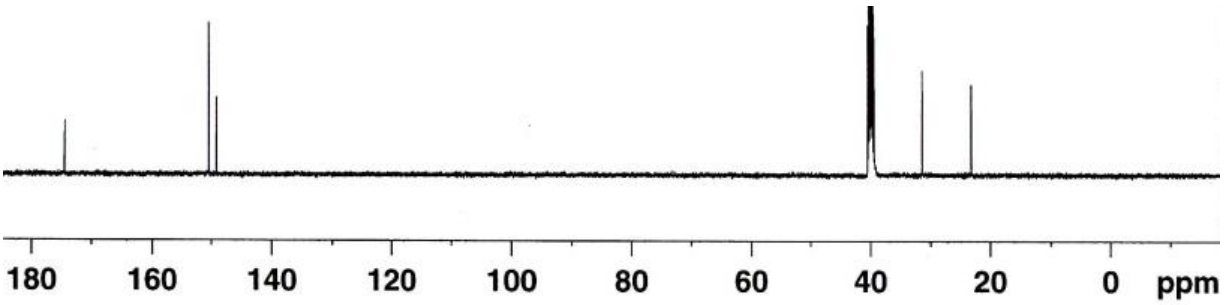
^{1}H NMR spectrum of **CyCo6** in DMSO-d_6



^{13}C NMR spectrum of **CyCo6** in DMSO-d_6



^{1}H NMR spectrum of **CyCo4** in DMSO-d_6



^{13}C NMR spectrum of **CyCo4** in DMSO-d_6

Derivation of equations for the pH sensitivity of self-assembling acid and base monomers:

We consider a system of two molecules, an acid **AH** and a base **B**, that assemble in aqueous solution. The deprotonated (i.e., negatively charged) form of **AH** is designated as $\mathbf{A}^{\ominus}$, and the protonated (i.e., positively charged) form of **B** is designated as $\mathbf{BH}^{\oplus}$.

If the neutral forms of these molecules assemble with a 1:1 stoichiometry, then we can define a solubility product for these monomers that is the product of the concentrations of the free (uncharged) monomers that coexist in solution with the supramolecular assembly. That is,

$$K_{sp} = [\mathbf{AH}][\mathbf{B}] \qquad \text{(Eq. 1)}$$

We can write the Henderson-Hasselbalch equations for both monomers as

$$\mathrm{pH} = \mathrm{p}K_{\mathrm{AH}} + \log\frac{[\mathbf{A}^{\ominus}]}{[\mathbf{AH}]} \quad \text{and} \quad \mathrm{pH} = \mathrm{p}K_{\mathrm{BH}^{\oplus}} + \log\frac{[\mathbf{B}]}{[\mathbf{BH}^{\oplus}]},$$

where $\mathrm{p}K_{\mathrm{AH}}$ is the pK_a of **AH** and $\mathrm{p}K_{\mathrm{BH}^{\oplus}}$ is the pK_a of the conjugate acid of **B**. Using these equations, the concentrations of the ionized forms of these molecules can be related to the concentrations of their neutral forms,

$$[\mathbf{A}^{\ominus}] = [\mathbf{AH}] \times 10^{\mathrm{pH}-\mathrm{p}K_{\mathrm{AH}}} \quad \text{(Eq. 2)},$$

$$\left[\mathbf{BH}^{\oplus}\right] = [\mathbf{B}] \times 10^{\mathrm{p}K_{\mathrm{BH}^{\oplus}}-\mathrm{pH}} \text{ (Eq. 3)}.$$

Here, to simplify our derivation, we consider only samples with equal molar amounts of each monomer. Because the supramolecular assemblies and the sample as a whole both have a 1:1 stoichiometry of **AH** and **B**, the concentrations of the two monomers free in solution will also be equal. That is, [**AH**] + $[\mathbf{A}^{\ominus}]$ = [**B**] + $[\mathbf{BH}^{\oplus}]$. Using Eq. 2 and Eq. 3, we can rewrite this equality as:

$$[\mathbf{AH}](1 + 10^{\mathrm{pH}-\mathrm{p}K_{\mathrm{AH}}}) = [\mathbf{B}](1 + 10^{\mathrm{p}K_{\mathrm{BH}^{\oplus}}-\mathrm{pH}}) \qquad \text{(Eq. 4)}$$

Using Eq. 1 to eliminate [**B**] from Eq. 4, and solving for [**AH**] we obtain,

$$[\mathbf{AH}] = \sqrt{\frac{K_{\mathrm{sp}}(1+10^{\mathrm{p}K_{\mathrm{BH}^{\oplus}}-\mathrm{pH}})}{(1+10^{\mathrm{pH}-\mathrm{p}K_{\mathrm{AH}})}}} \ . \qquad \text{(Eq. 5)}$$

The total concentration of the neutral and ionized species of **AH** in solution can then be written as,

$$[\mathbf{AH}] + [\mathbf{A}^{\ominus}] = \sqrt{K_{\mathrm{sp}}(1 + 10^{\mathrm{p}K_{\mathrm{BH}^{\oplus}}-\mathrm{pH}})(1 + 10^{\mathrm{pH}-\mathrm{p}K_{\mathrm{AH}}})} \ . \qquad \text{(Eq. 6)}$$

We define the Normalized Fraction Assembled, or NFA, as the relative fraction of monomers assembled at a particular pH as compared to the fraction of monomers assembled at the pH of maximum assembly, which is midway between $\mathrm{p}K_{\mathrm{AH}}$ and $\mathrm{p}K_{\mathrm{BH}^{\oplus}}$ (pK_{ave}). That is,

$$\mathrm{NFA} = \frac{\mathbf{A}_{\mathrm{tot}} - \sqrt{K_{\mathrm{sp}}(1+10^{\mathrm{p}K_{\mathrm{BH}^{\oplus}}-\mathrm{pH}})(1+10^{\mathrm{pH}-\mathrm{p}K_{\mathrm{AH}}})}}{\mathbf{A}_{\mathrm{tot}} - \sqrt{K_{\mathrm{sp}}}(1+10^{\mathrm{p}K_{\mathrm{ave}}-\mathrm{p}K_{\mathrm{AH}}})} \ , \qquad \text{(Eq. 7)}$$

where $\mathbf{A}_{\mathrm{tot}}$ is equal to the *total* concentration of **AH** in the sample, in neutral and ionized forms, and as free monomers and in supramolecular assemblies. The positive values of Eq. 7, ranging from 0 to 1, represent physically meaningful solutions to the NFA; negative values indicate samples with completely unassembled monomers.

We note that a similar equation is obtained if the ionized species of **AH** and **B** form the supramolecular assembly. In which case $K_{\mathrm{sp}} = [\mathbf{A}^{\ominus}][\mathbf{BH}^{\oplus}]$, and

$$\mathbf{NFA} = \frac{\mathbf{A}_{\mathrm{tot}} - \sqrt{K_{\mathrm{sp}}\left(1+10^{\mathrm{pH}-\mathrm{p}K_{\mathrm{BH}^{\oplus}}}\right)\left(1+10^{\mathrm{p}K_{\mathrm{AH}}-\mathrm{pH}}\right)}}{\mathbf{A}_{\mathrm{tot}} - \sqrt{K_{\mathrm{sp}}}(1+10^{\mathrm{p}K_{\mathrm{AH}}-\mathrm{p}K_{\mathrm{ave}}})} .$$

Examples of curves generated using Eq. 7:

For the experimental data presented in the main text, **CyCo6**+**CyCo4** is **AH**; **TAP** is **B**. The $\mathrm{p}K_{\mathrm{AH}}$ of **CyCo6** and **CyCo4** are equal to the $\mathrm{p}K_{\mathrm{BH}^{\oplus}}$ of **TAP**, which is set here to 7.4. Experiments were carried out at pH 7.4 with total concentration **TAP** of 35 mM and total **CyCo6**+**CyCo4** concentration of 35 mM, exhibiting a free monomer concentration of 15 mM in **TAP** and 15 mM in **CyCo6**+**CyCo4**. The concentrations of the *neutral* species of **AH** and **B** are therefore each 7.5 mM at the pH of maximum assembly. Thus, $\mathbf{A}_{\mathrm{tot}}$ = 35 mM, K_{sp} = (7.5 mM)^2, pK_{ave} = pK_{AH}, and Eq. 7 reduces to:

$$\mathbf{NFA} = \frac{35 - 7.5\sqrt{(1+10^{7.4-\mathrm{pH}})(1+10^{\mathrm{pH}-7.4})}}{20},$$

which gives the green curve shown in Figures 1a and 4 when plotted versus pH.

If **AH** and **B** are not pK_{a} matched (i.e., $\mathrm{p}K_{\mathrm{AH}} < \mathrm{p}K_{\mathrm{BH}^{\oplus}}$), but the neutral monomers assemble with the same K_{sp}, then the **NFA** of solutions containing 35 mM in each monomer (neutral and ionized, free and assembled), is given by:

$$\mathbf{NFA} = \frac{35 - 7.5\sqrt{(1+10^{\mathrm{p}K_{\mathrm{BH}^{\oplus}}-\mathrm{pH}})(1+10^{\mathrm{pH}-\mathrm{p}K_{\mathrm{AH}}})}}{35 - 7.5(1+10^{\mathrm{p}K_{\mathrm{ave}}-\mathrm{p}K_{\mathrm{AH}}})}.$$

For example, if pK_{ave} of **AH** and **B** is still 7.4, but their ΔpK_{a} is 5 (i.e., $\mathrm{p}K_{\mathrm{AH}}$ = 4.9 and $\mathrm{p}K_{\mathrm{BH}^{\oplus}}$ = 9.9), then the **NFA** curve is given by:

$$\mathbf{NFA} = \frac{35 - 7.5\sqrt{(1+10^{9.9-\mathrm{pH}})(1+10^{\mathrm{pH}-4.9})}}{35 - 7.5(1+10^{2.5})},$$

which is the red curve shown in Figure 1a.